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THE OXIDASE OF RHUS DIVERSILOBA *

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THE PRESENCE OF THE OXIDASE IN THE PLANT AND ITS RELATION TO THE PLANT POISON

It has often been noticed that when a plant of Rhus diversiloba, T. and G., is injured, the exuding sap rapidly becomes brown and hardens into a black, shiny, varnish-like substance. This phenomenon becomes more interesting when it is realized that the violently irritating properties of the fresh sap are completely lost during the formation of this resin-like body. This change in toxic properties may be readily followed in connection with the darkening of the sap, as the poisonous property of the freshly exuded sap is soluble in 95 per cent. alcohol, as well as its nontoxic black oxidation product. The poisonous and nonpoisonous alcohol-soluble substances may readily be separated, however, for the oxidation product is insoluble in xylol, whereas the toxic substance is soluble in xylol.

No evidence is here set forth to prove that the substance darkened is the poison. The darkening of the sap through oxidation is considered here merely as an indicator of the extent of the loss of toxic properties.

This darkening and loss of toxic properties seems to be mainly due to oxidation in the presence of moisture, as the following experiments indicate:

Sap from a fresh incision in a rhus plant was thinly coated for about 1 sq. cm. on the inside of test tubes, ¾ by 6 inches in size, which had been thoroughly cleaned in concentrated sulphuric acid saturated with potassium dichromate. The tubes were now filled with gases as follows: 2 with oxygen; 2 with hydrogen from the reaction of zinc and sulphuric acid, purified by bubbling successively through 10% lead nitrate, 10% silver nitrate, and alkaline pyrogallol; 2 with carbon dioxid; 2 with nitrogen secured by shaking air with alkaline pyrogallol in a Hempel pipet; and 2 with air. These gases, before entrance to the test tubes, were dried by passing through calcium-chlorid tubes. One test tube of each gas had 1 c.c. of distilled water placed in it. The tubes were finally corked with rubber corks, sealed with paraffin, and kept at the

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temperature of the surrounding air, which at this time ranged between 13 and 19 C. The results are as follows:

In dry air, the sample did not appreciably darken.
In moist air, the sample darkened after 5 hours.
In dry oxygen, the sample did not appreciably darken.
In moist oxygen, the sample darkened after 3 hours.
In dry hydrogen, the sample did not appreciably darken.
In wet hydrogen, the sample darkened after 2 days.
In dry carbon dioxid, the sample did not appreciably darken.
In wet carbon dioxid, the sample darkened very slowly after 2 days.
In dry nitrogen, the sample did not appreciably darken.
In wet nitrogen, the sample darkened after 2 days.

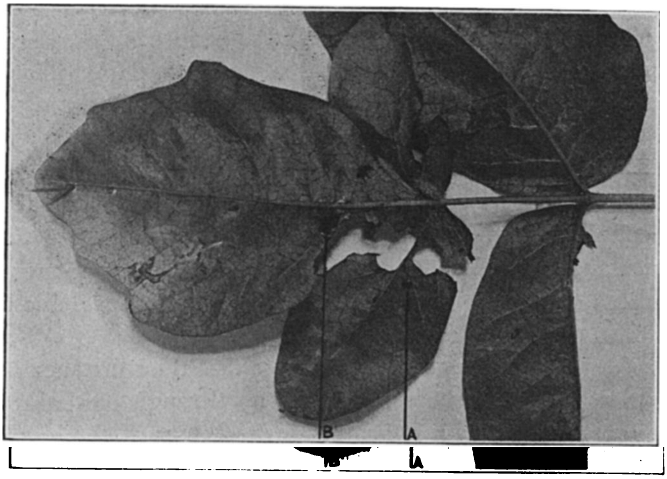

Fig. 1. Rhus-diversiloba leaf. A and B indicate the resinified poison on insect-injured leaf.

The absorption of oxygen during the drying of the sap can be readily followed by its increase in weight.

Fresh sap spread in a thin layer within a beaker was dried in a desiccator in an atmosphere of hydrogen until constant weight had been attained (about 12 hours). The sap-coated beaker thus freed from moisture was placed in a damp place for several days. At the expiration of this time the beaker and contents were again placed in a desiccator until constant weight was secured. An increase in weight of approximately 1%, together with the hardening and darkening of the sap, had occurred. The increase in weight, however, did not cease after several days, but continued for at least a month.

By the use of a combustion furnace it was found that fresh sap when dried contains a lower percentage of oxygen than a specimen which has been exposed to the air for a week. All the other elements remain constant. The increase in weight, therefore, is due to oxidation.

Experiments were next conducted to determine the effect of different temperatures upon the darkening of the expressed sap.

Test tubes, the same size as those described and similarly cleaned, were thinly coated inside, over about 1 sq. cm., with fresh sap. One cubic centimeter of distilled water was now placed in each test tube, and rubber corks introduced. They were then individually subjected to the following temperatures:

At 5 C. a sample did not appreciably darken.
At 20 C. a sample darkened after 4 hours.
At 30 C. a sample darkened after 4½ hours.
At 40 C. a sample darkened after 5 hours.
At 50 C. a sample darkened after 7 hours.
At 60 C. a sample darkened after 24 hours.
At 80 C. a sample did not appreciably darken.

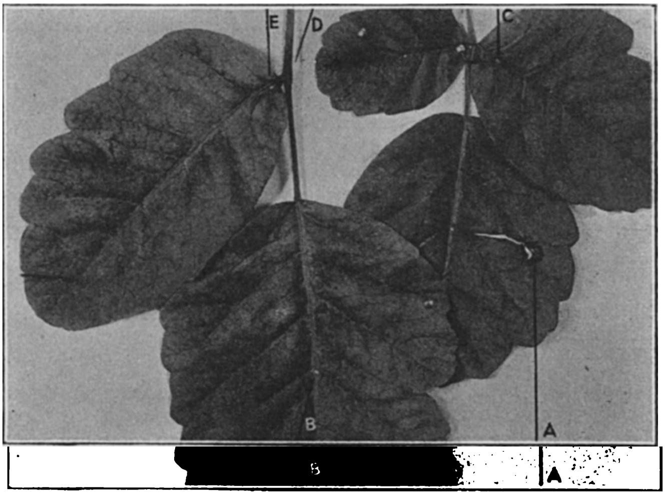

Fig. 2. Rhus-diversiloba leaves. A indicates resinified poison on injured part; D, resinified poison at base of leaflet; B, C, E, scale insects (Aspidiotis). A case of natural immunity. The scale insects were identified by Mr. G. A. Coleman.

These experiments indicate that the optimum temperature for the oxidation of the sap by the enzyme lies somewhere between 5 and 30 C. and nearer 20 than 30 C. The total range of its activity lies between 5 and 80 C.

Atkins[1] has observed that a browning of expressed sap is invariably associated with the presence of an oxidase. (The brown saps which Atkins investigated were those from: Hedera helix, Syringa vulgaris,

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[1] Sc. Proc. Roy. Dublin Soc., 1913, 14, p. 144.

Magnolia acuminata, Catalpa bignonioides, Fraxinus oxyphylla, Fraxinus excelsior, and Helianthus multiflorus). This phenomenon is analogous to the changing of the sap of the lactree, Rhus vernicifera, when exposed to the air for some time. It is transformed into a very beautiful black varnish, which the Chinese and Japanese use for varnishing their furniture.[2] It is not surprising then that an oxidase should be found in Rhus diversiloba.

Similar oxidizing enzymes have been found by Bertrand[3] in plants destitute of chlorophyl; for example, 2 mushrooms, Boletus euridus and Agaricus sanguineus. The enzyme, then, is not necessarily a dependent of chlorophyl, and when present in green plants it might be expected to continue its work of oxidation in the leaves after they have become destitute of chlorophyl. The death of the protoplasm, as well as the presence of conditions unfavorable to the normal metabolism of the cell, might not improbably be followed by increased oxidase activity. This has been found true in the case of insect injury to Rhus diversiloba, abscission of the mid-rib in the leaf, severe twisting of the stem, and a number of other instances. It has been noticed when mulberry trees were cut back too frequently that an abnormal yellow color and crinkled appearance resulted in the leaves; Suzuki[4] investigated this to find that an excessive production of oxidases had taken place in such yellow areas. This he attributed to the lack of proper nutrition of rapidly growing tissues. Woods[5] observed a similar phenomenon in the 'mosaic disease' of tobacco plants which had been cut back.

Bunzel[6] investigated the oxidase content of the normal and 'curly top' diseased leaves of a sugar beet. He found that the diseased leaves had an oxidase content 2 or 3 times greater than that of healthy and normally developed ones. The roots of the two kinds of plants showed no marked differences however.

When the mature leaves of Rhus diversiloba are green, they give no oxidase reaction with gum guaiac.

For this experiment a finely chopped leaf was placed in a test tube with 10 c.c. of water heated to 25 C. Enough alcoholic solution of guaiac (strength about 1:60) to cause a turbidity was added. No blue color appeared in this mixture before it did in an aqueous check solution of the same temperature and concentration of guaiac. The failure of the green leaves to give this oxidase reaction may have been due to the presence of tannin or gallic acid in them, as these substances have been shown by Hunger[7] to interfere with the guaiac test. The presence of tannin, gallic acid, or gallotannin was indicated by the reagent of Fletcher and Allen[8] (0.05 gm. of pure potassium ferricyanid dissolved in 50 c.c. of water, and an equal volume of concentrated ammonia water added). This indicator produces a red coloration with tannin, gallic acid, or gallotannic acid in solution, being so sensitive that a drop of the indicator will detect 1 part of tannin in 10,000 parts of water.

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[2] Yoshida, Jour. Chem. Soc. Transactions, 1883, 43, p. 472. Yoshida and Korschet, As. Soc. Japan, 12, p. 182.

[3] Compt. rend. Acad. d. sci., 1894, 118, p. 1215. Bull. Soc. chim., Paris, 1894, 11, p. 717. Compt. rend. Acad. d. sci., 1895, 120, p. 266; 121, p. 166; 1896, 122, p. 1132. Bull. Soc. chim., Paris, 1896, 15, p. 791. Compt. rend. Acad. d. sci., 1896, 122, p. 1215. Bull. Soc. chim., Paris, 1896, 15, p. 793. Jour. Pharm. et Chim., 1896, 3, p. 607. Compt. rend. Acad. d. sc., 1896, 123, p. 463. Ann. Chim. et Physique, 1897, 12, p. 115. Bull. Soc. chim., Paris, 1897, 17, p. 753. Compt. rend. Acad. d. sc., 1897, 124, pp. 1032, 1355. Jour. Pharm. et Chim., 1897, 5, p. 545. Arch. Agronom., 1897, 23, p. 285. Bull. Soc. chim., Paris, 1897, 17, p. 577. Ann. de l'Inst. Pasteur, 1902, 16, p. 179. Compt. rend. Acad. d. sci., 1907, 145, p. 340. Bull. Soc. chim., Paris, 1908, 145, p. 1352. Compt. rend. Soc. de biol., 1896, 48, p. 811; 1895, 47, p. 579.

[4] Bull. Agr. College Tokyo, 1900, 4, p. 167.

[5] Woods, U. S. Dept. Agr. Bull., 18, 1902.

[6] U. S. Dept. Agr., Bur. Plant Ind. Bull., 277, 1913.

A positive test for oxidase may be secured with guaiac after the inhibitor has been removed. A rapid method for separating the inhibiting substance and enzyme is that employed by Aso[9] for tannin. The precipitation of the enzyme may be effected by pouring the expressed sap into 6 volumes of 95% alcohol. The precipitate when redissolved in water gives the guaiac reaction. This color is destroyed by addition of the filtrate.

In autumn when the leaves become red and yellow, positive results are obtained for oxidase with gum guaiac and the leaves contain no tannin. (If sufficient tannin was added to the solution before testing, the guaiac test became negative.)

The alcoholic solution made from the autumnal leaves is less toxic than a similar solution of equal concentration from the normal leaves when applied to histologically corresponding areas of skin of a very sensitive person. Of the autumnal leaves the red are less toxic than the yellow, and when the leaves have finally withered and fallen they are nontoxic. I am indebted to Dr. Edward von Adelung, who is very sensitive to the poison, for the determination of these differences in toxicity.

This gradual loss of toxicity of the leaves through oxidation has led several observers to consider the poison volatile. For instance, Mackie,[10] writing on the value of oak leaves for forage, says: "It would seem that the irritating and poisonous oil of poison oak is volatile at a comparatively low temperature. In gathering the specimens the writer was badly poisoned even though gloves were worn; yet after drying at ordinary room temperature, and the leaves pressed into the mill with bare hands, no poisoning effects followed." That there is no volatile poison in the plant was known to Dr. von Adelung,[11] and this has been verified by further experiments in a more recent article.[12] This loss of toxicity is therefore due to the oxidation of the poison and the loss of fluidity of the sap.

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[7] Ber. die Bot. Ges., 1901, 19, p. 648.

[8] Chemical News, 29, pp. 167 and 189.

[9] Botanical Magazine, Tokyo, 1890, 14, No. 166.

[10] California Exper. Sta., Bull. 150, 1903.

[11] Arch. Int. Med., 1913, 2, p. 184.

[12] McNair, Jour. Infect. Dis., 1916, 19, p. 429.

THE PREPARATION OF THE ENZYME EXTRACT

The plant leaves were used as sources of the ferment and the following method was pursued in its preparation: Fresh green leaves were washed in many changes of water, finally in distilled water. After draining, the leaves were finely cut with a meat chopper. The finely chopped material was mixed with distilled water, glycerin, and a small amount of chloroform, and allowed to soak for 48 hours. The mixture was now filtered through cotton and the filtrate reserved. (As this solution was made from green leaves it contained tannin but not sufficient to inhibit the guaiac reaction. In fact for some reason not investigated the addition of tannin to this solution did not interfere with the guaiac test.)

The constants of the filtered enzyme solution were as follows:

Density—23 degrees Baume (sp. gr. 1.190) at 18.5 C.
Glycerol content—73%, or 270.37 parts by weight of glycerin for each 100 parts of water.
Ash—1.666 gm. per liter, or 0.0016%.*

* Tannin, 0.0012 per cent, estimated by a comparison of the amount of ash with the amount of ash and tannin obtained by Mackie[10] from water-free Rhus-diversiloba leaves. His analysis yielded 9.15 per cent. ash, 7.24 per cent. protein, 26.56 per cent. fiber, 6.79 per cent. tannin (Günther), 43.39 per cent. nitrogen-free extract, and 6.87 per cent. ether extract.

When tested according to the "Qualitative Analysis" of Morgan, 1907, p. 239, the ash gave positive indications for iron, aluminum, calcium, magnesium, silica, sodium, potassium, and phosphorus.

This ash analysis shows the oxidase to be unlike that of Bertrand obtained from Rhus vernicifera, which contained manganese. On the other hand, Sarthou[13] found the oxidase of Schinus molle (a tree of the same family as Rhus diversiloba, namely, the anacardiaceae) to contain iron, calcium, sodium, but no manganese.

This enzyme solution was used in the experiments which follow.

RELATION OF THE MANNER OF ACTION OF THE ENZYME TO THE CHEMICAL COMPOSITION OF THE POISON

Emil Fisher[14] has evolved the hypothesis that in the majority of ferment actions there exists a stereochemic relation between the acting substance and the body acted upon. According to him, it is necessary that the ferments and the substances they act upon, have like geometric structures.

Any clue as to the nature of the substances acted upon by the oxidase of Rhus diversiloba may therefore be expected to throw some light upon the nature of the poison itself. So far all that has been done in the direction of defining the nature of this enzyme has been the discovery of its action upon the poison and the circumstances of reaction, etc., favoring this action. The oxidation of the poison by its enzyme is favored by alkalies — for example, potassium hydroxid and sodium hydroxid — and may be greatly retarded, if not inhibited, by the presence of acids.

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[13] Jour. Pharm. et Chim., 1900, 11, p. 482; 12, p. 583; 1901, 13, p. 464.

[14] Ber. d. deutsch. Chem. Ges., 1894, pp. 2071, 2985, 1429, 3499.

Enzymes are considered as catalyzers and merely accelerate an oxidation that would take place in their absence at a slower rate. Attention was therefore directed to readily oxidizable substances. Of bodies most readily oxidized those of the aromatic series seem to be more susceptible than those of the aliphatic series. As the oxidation of polyphenols is considerably increased by small quantities of alkali,[15] it was thought the enzyme might be a phenolase. Consequently its action was tested with the diphenols, hydroquinon, pyrocatechin, resorcin, and the triphenols, phloroglucin and pyrogallol.

The Preparation of Hydrochinon Solution and the Colorimetric Method of Testing.—One gram of hydrochinon was weighed rapidly so as to prevent oxidation as much as possible. The weighed phenol was placed in a 250-c.c. Erlenmyer flask together with 99 c.c. of distilled water. Three such solutions were made with hydrochinon. From 5 to 15 c.c. of the enzyme solution were added to individual phenol solutions.

Some of the enzyme solution was boiled for 5 minutes (distilled water being added to keep the volume constant). After boiling and consequent cooling to room temperature this destroyed enzyme solution was added in varying amounts to 1% hydrochinon solutions. The three phenol solutions, one normal, another with the added enzyme, and the third with the destroyed enzyme, were subjected to a temperature varying from 14 to 15 C. for 3.5 hours.

As hydrochinon has a strong tendency to oxidize in the air without any accelerating enzyme, check solutions were used in which equal amounts of the substance tested were placed without the enzyme. In order to decide whether the reactions were due to enzymatic or to an ordinary chemical change caused by the glycerin plant extract, checks were used in which an equivalent amount of enzyme solution was boiled before being added to the phenol solution. As the reaction was not equally accelerated by the boiled enzyme, it was concluded that its diminution in activity was caused by the destruction of a thermolabile oxidizing agent. The results are recorded in Table 1.

TABLE 1

FINAL COLORS AS ESTIMATED BY A DUBOSCQ COLORIMETER

	No Enzyme (Depth, mm.)	5 c.c. Enzyme Solution (Depth, mm.)	10 c.c. Enzyme Solution (Depth, mm.)	15 c.c. Enzyme Solution (Depth, mm.)
Normal enzyme	20	23.5	26.5	31.0
Boiled enzyme	20	21.0	22.5	25.0
Difference in depth of color	0	2.5	4.0	6.0

The results obtained in a series of experiments were not very satisfactory and therefore the Van Slyke apparatus was tried.

The Method of Testing the Oxidase with the Van Slyke Apparatus.—The apparatus used was that described by Van Slyke.[16] The structure of the apparatus and the manner in which it was set up are apparent from the accompanying cut and photograph.

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[15] Colleman and Walker, Organic Chemistry, p. 409.

[16] Jour. Biol. Chem., 1912, 12, p. 275.

As the Van Slyke apparatus does not appear to have been hitherto employed for the study of oxidases, directions for its manipulation follow:

1. The admission of the aromatic compound: Water from F fills the capillary leading to the Hempel pipet and also the other capillary as far as C. Into A one pours a volume of the phenolic solution to be oxidized sufficient to fill D up to the 20-c.c. mark (the excess may be drawn off by the cock d). One then closes a and turns c and f so that D and F are connected.

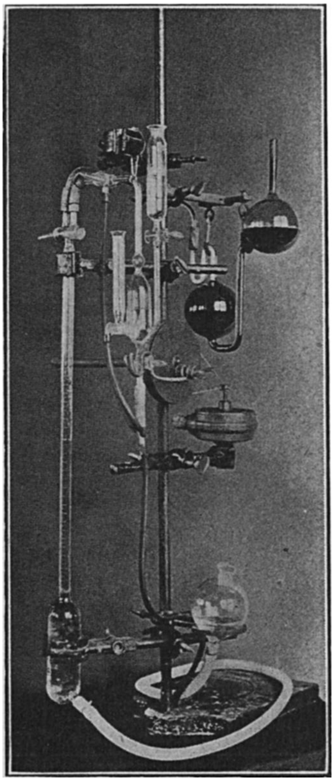


Fig. 3. The complete Van Slyke apparatus.

2. The admission of the enzyme solution and the oxidization of the aromatic compound: One places the enzyme solution in B. The admission of the desired amount of enzyme solution into the oxidizing bulb D is accomplished by opening the cock between B and D. The oxidizing bulb is now connected with the motor, as shown in the photograph, and shaken for the determined time.

3. The determination of the amount of oxygen absorbed by the aromatic compound: The reaction being completed, all the gas in D is displaced into F by distilled water from A and a buret reading taken by closing the cock f and lifting the bulb attached to the buret, until the meniscus in the buret and that in the bulb are on the same horizontal plane. After the reading is taken, the gas is forced over from F into the absorption pipet by merely opening f and lifting the buret bulb. The driving rod is then connected with the pipet by lifting the hook from the shoulder of d and placing the other hook, on the opposite side of the driving rod, over the horizontal lower tube of the pipet. The latter is then shaken by the motor for 5 minutes, which, with any but almost completely exhausted alkaline pyrogallol solutions, completes the absorption of the residual oxygen. The residual, gas mostly, nitrogen is then measured in F.

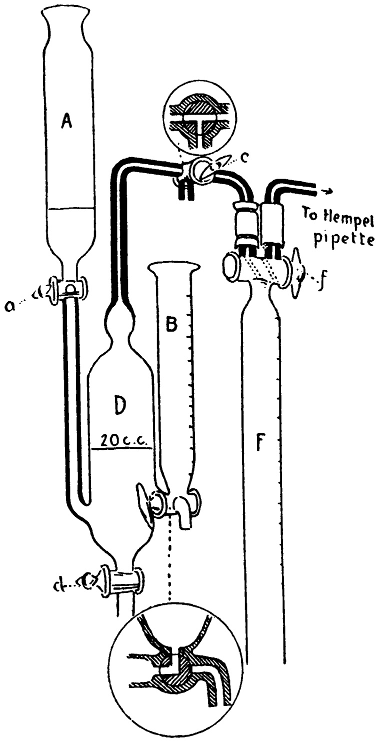
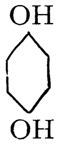
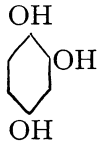


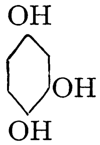
Fig. 4. Oxidizing bulb and connections in detail. The Van Slyke apparatus.

The alkaline solution of pyrogallol used in the Hempel pipet, is prepared by mixing together directly in the absorption pipet 5 gm. of pyrogallol dissolved in 15 c.c. of water, and 120 gm. of potassium hydroxid dissolved in 80 c.c. of water.

When tried with 3 isomeric diphenols, namely,







hydroquinon, pyrocatechin, and resorcin,

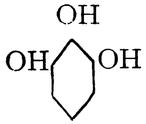
in the Van Slyke apparatus, it was seen that the quantity of gas absorbed was almost nil for the metadiphenol (resorcin), while the paradiphenol (hydroquinon) oxidized very strongly. The ortho diphenol (pyrocatechin) maintained an intermediate position.

TABLE 2
RESULTS OF THE TEST OF THE ENZYME WITH THE VAN SLYKE APPARATUS

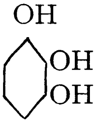
2% hydroxy-phenol (c.c.)	Enzyme Solution (c.c.)	Oxidizer Shaken (min.)	Gas (c.c.)	Pipet Shaken (min.)	Gas (c.c.)	Difference in Amount of Gas (c.c.)	Accelera-tion
			Hydrochinon				
20	2	10	34.7	5	29.6	5.1	0.8
20	0	10	34.5	5	28.6	5.9	
			Pyrocatechin				
20	2	10	35.4	5	29.9	5.5	0.6
20	0	10	34.1	5	28.0	6.1	
			Resorcin				
20	2	10	34.8	5	28.7	6.1	0.1
20	0	10	34.4	5	28.2	6.2	

Room temperature throughout the experiment 23.5–24 C.
Barometric pressure throughout the experiment 752 mm.

The action of the enzyme was tested on 2 isomeric triphenols, namely, phloroglucin, in which all the hydroxyls are in the meta position (symetrically placed), and pyrogallol, with its consecutive hydroxyls. It was found that phloroglucin did not oxidize, while pyrogallol absorbed oxygen rapidly.



phloroglucin



pyrogallol

TABLE 3

RESULTS OF TEST OF THE ENZYME WLTH PHLOROGLUCIN AND PYROGALLOL

2% hydroxy-phenol (c.c.)	Enzyme Solution (c.c.)	Oxidizer Shaken (min.)	Gas (c.c.)	Pipet Shaken (min.)	Gas (c.c.)	Difference in Amount of Gas (c.c.)	Accelera-tion
			Pyrogallol				
20	2	10	34.1	5	28.8	5.3	0.3
20	0	10	34.1	5	28.5	5.6	
			Phloroglucin				
20	2	10	34.5	5	28.3	6.2	0.0
20	0	10	34.8	5	28.6	6.2	

Room temperature throughout the experiment 23.5-24 C.
Barometric pressure throughout the experiment 752 mm.

From the foregoing it would seem that the enzyme is a soluble ferment accelerating the oxidation of bodies of the phenol series, which possess at least 2 hydroxyl groups, when these groups occupy the para or ortho position.

The experiments recorded in Table 4, in which the amount of substrate is constant, show that this enzyme obeys the rule expressed by the formula $F.t = \text{const.}$ In other words, the acceleration of oxidation is directly proportional to the concentration of the ferment, and the time required to achieve a given amount of oxidation is inversely proportional to the amount of ferment present.[17]

TABLE 4

RESULTS OF THE TEST OF THE ENZYME WITH THE VAN SLYKE APPARATUS

2% Hydro-chinon (c.c.)	Enzyme Solution (c.c.)	Oxidizer Shaken (min.)	Gas (c.c.)	Pipet Shaken (min.)	Gas (c.c.)	Difference between Amounts of Gas (c.c.) (observed equivalent constant)	Constant Calculated $ft = k$ $k = 30$
20	1	30.0	34.4	5	28.4	6.0	30
20	2	15.0	34.3	5	28.8	5.5	30
20	3	10.0	34.9	5	28.6	6.3	30
20	4	7.5	35.1	5	28.6	6.5	30
20	5	6.0	35.6	5	29.1	6.5	30
	f	t				k	k

Room temperature throughout experiment 18 C.
Barometric pressure throughout experiment 759 mm.

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[17] Hedin, Jour. Phvsiol., 1905, 32, p. 468; 1906, 34, p. 370. Hoppe, Seyler's Ztschr. f. physiol. Chem., 1908, 57, p. 471.

The enzyme solution was found to accelerate the oxidation of other bodies of the aromatic series, for example those containing amin groups, as paramidophenol $C_6H_4.OH.NH_2(1,4)$ and paraphenylenediamin $C_6H_4.NH_2NH_2(1,4)$.

TABLE 5

DEPENDENCE OF RAPIDITY OF OXIDATION UPON THE CONCENTRATION OF ENZYME

2% Hydrochinon (c.c.)	Enzyme Solution (c.c.)	Oxidizer Shaken (min.)	Gas (c.c.)	Pipet Shaken (min.)	Gas (c.c.)	Difference between Amounts of Gas (c.c.) (4-6)	Comparative Amount of Absorption (7-n)	Calculated*
20	0	10	34.6	5	27.9	6.7	0	0
20	1	10	33.7	5	28.5	5.2	1.5	1.34
20	2	10	34 5	5	29.5	5.0	1.7	1.90
20	3	10	34.5	5	29.9	4.6	2.1	2.32
20	4	10	33 0	5	28.9	4.1	2.6	2.68
20	5	10	33.9	5	30.5	3.4	3.3	3.00

* Calculated 1.34 $\sqrt{\text{amount of enzyme}}$.
Room temperature throughout experiment 19 C.
Barometric pressure throughout experiment 752 mm.

TABLE 6

DEPENDENCE OF THE AMOUNT OF OXIDATION UPON THE TIME OF EXPOSURE TO THE OXIDASE

2% Hydrochinon (c.c.)	Enzyme Solution (c.c.)	Oxidized Shaken (min.)	Gas (c.c.)	Pipet Shaken (min.)	Gas (c.c.)	Difference in Amounts of Gas	
						c.c. Observed	c.c. Calculated*
20	1	5	33.9	5	27.5	6.4	6.44
20	1	10	33.9	5	27.8	6.1	6.06
20	1	15	34.1	5	27.7	5.4	5.42
20	1	20	33.8	5	29.3	4.5	4.52

* $6.57 - 0.128 \left[\frac{t}{5}\right]^2$
Room temperature throughout experiment 25.5 C.
Barometric pressure throughout experiment 754 mm.

DISCUSSION OF RESULTS

From the tabulated experiments the relations required by chemical dynamics for the simplest cases of catalytic reactions are found to be fulfilled by the action of the rhus enzyme on 2% hydrochinon: (1) approximate proportionality between the concentration of the enzyme and the velocity of the reaction (Duboscq colorimeter experiment); and (2) the validity of the rule $Ft = k$, indicating that the time required to achieve a given amount of oxidation is inversely proportional to the amount of ferment present.

It is interesting to note that the normal oxidation when combined with the accelerated oxidation is proportional to the square root of the amount of enzyme when the temperature is 25.5 C. This result is the same as that which Slowtzoff[18] obtained with potato laccase. There is a remarkable difference between this result and that obtained at 19 C. At 19 C. the result of the combined oxidations is more nearly proportional to the square of the amount of time. This difference seems unexplainable when compared with the rate of most chemical reactions, which is doubled or trebled for a rise of 10 degrees. This general rate may be applicable to the normal oxidation of hydrochinon separately, but the acceleration of this particular reaction due to the enzyme is greater at 19 than at 25 C., as shown in the beginning of this paper. At moderate temperatures the temperature coefficients of enzyme reactions are approximately the same as those of chemical reactions in general, but at temperatures in the neighborhood of 0 C.; the rate of change of the velocity constant with the temperature is often abnormally high, while at temperatures in excess of the optimum, that is, involving destruction of the enzyme, the velocity constant rapidly approaches zero. In the present instance the optimum temperature would appear to lie below 20 C.

THERAPEUTIC ACTION OF THE ENZYME SOLUTION

As the enzyme has the power to change the poison to a nontoxic substance while on the plant, it was thought that it might change the poison to a nontoxic substance when on the skin and thus be a remedy for rhus dermatitis. Experiments were conducted for me by Dr. Edward von Adelung to ascertain the value of the enzyme solution as: (1) Poisonous or not; (2) a preventative of rhus dermatitis; (3) remedy. The following results were attained: (1) The enzyme solution did not produce dermatitis tho rubbed briskly into the skin. (2) When mixed with rhus poison in alcoholic solution it did not destroy the poison. (The enzyme is active in 50% alcohol.) (3) It has no remedial value.

These results are as might be expected, for the enzyme is present in the expressed sap of the plant, and yet, when the expressed sap is rubbed on sensitive skin, dermatitis results. (Positive results for the presence of the enzyme in the sap may be obtained by mixing the sap with 6 times its volume of 95% alcohol. Filtering, dissolving the precipitate in distilled water, and testing with guaiac solution as on Page 6 of this thesis.) The enzyme is colloidal and will not pass through a semipermeable membrane, for example, parchment paper.

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[18] Ztschr. f. physiol. Chem., 1900, 31, p. 227.

The poison, on the other hand, is not colloidal as when in ethylacetate solution the poison will pass through semipermeable membrane. Another evidence of the poison's ability to penetrate a semipermeable membrane is seen in its ability to penetrate the skin in the production of rhus dermatitis.[12] It is quite probable that the enzyme cannot penetrate the semipermeable skin and thus is unable to follow up and destroy the poison. Failure to show remedial properties in this enzyme solution, apart from its inability to penetrate normal skin, may be due to a matter of dilution. There are, however, oxidizers in the skin which seem to be incapable of the rapid destruction of the poison, for example, the leukocytes and nucleoprotein.[19]

SUMMARY

The browning of the sap of Rhus diversiloba is due to oxidation.

This oxidation results in the total loss of the irritating poisonous properties of the sap.

This oxidation is greatly accelerated naturally by an enzyme.

The oxidase accelerates the transfer of molecular oxygen to phenols, and aromatic amins.

The oxidase accelerates the oxidation of ortho- and para-isomeric phenols more greatly than metaphenols.

The oxidizing power of the enzyme is accelerated by dilute alkalies.

The oxidizing power of the enzyme is retarded by dilute acids.

The oxidizing power of the enzyme is destroyed when boiled for a short time in aqueous solution.

Rhus diversiloba oxidase is probably similar to that isolated from Rhus vernicifera by Yoshida and Bertrand. (Stevens and Warren[20] observed a thermolabile enzyme in Rhus vernix, probably an oxidase, which gave characteristic reactions with guaiac, alpha naphthol, and guaiacol.) It is a soluble ferment accelerating the oxidation of readily oxidizable bodies, particularly those of the benzene series, which possess at least two groups hydroxyl or amin when these groups occupy the para or ortho position.

The oxidase apparently has no therapeutic value either as a preventive or as a cure for rhus dermatitis.

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[19] There is another phenomenon that tends to explain the therapeutic failure of the enzyme solution namely, that the body may form an anti-enzyme specific in its inhibitive action against rhus diversiloba oxidase. Gessard (Compt. rend. Acad. d. sc., 1903, 136, p. 631; Compt. rend. Soc. biol., 1903, 55, pp. 227, 637) obtained a serum capable of retarding the action of the oxidase of rhus vernicifera by the subcutaneous injection into a rabbit of preparations containing this enzyme.

[20] Am. Jour. Pharm., 1907, 79, p. 499.